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STATUS REPORT ON CRYOPRESERVATION OF HUMAN PLATELETS.(U)
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Status Report on Cryopreservation of Human Platelets

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release of ^{14}C labeled serotonin from platelets incubated with increasing concentrations of thrombin. Platelets frozen in polyolefin plastic bags, thawed and washed, labeled with ^{14}C serotonin, and washed again, responded to increasing concentrations of thrombin in a manner similar to that of fresh platelets; the response of platelets frozen in polyvinyl chloride plastic bags was about 90% that of platelets frozen in polyolefin plastic bags.

Fresh platelets and the frozen washed platelets labeled with ^{14}C serotonin are washed to remove the supernatant ^{14}C radioactivity prior to thrombin incubation. It may be that the removal of most of the residual DMSO from the frozen washed platelets contributed to our excellent results. The observations in this study on the response of previously frozen platelets in the release of ^{14}C labeled serotonin to increasing concentration of thrombin are the best observed here to date, and our previously poor *in vitro* results may have been related to the greater amount of residual DMSO in the platelets.

The release of ^{14}C labeled serotonin from platelets is the *in vitro* test now being used in this laboratory to evaluate the variables involved in the pre-freeze, freezing, and post-thaw-wash processing of human platelets. It is also used to study human platelets stored at 4°C and 22°C.

The significance of the ^{14}C serotonin release response with respect to platelet viability and function *in vivo* is now being studied.

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The equivalent of about 5 single units of platelets was isolated from each of eight normal volunteers by intermittent flow centrifugation using the Haemonetics Model 30 Blood Processor. The platelets were frozen with 6% DMSO in a single polyolefin plastic container in a -80 C mechanical freezer. In vitro freeze-thaw-wash platelet recovery values varied with the methods of measurement: 80% when the platelet counts were made by phase microscopy, 85% by Coulter counter, and 60% by the Technicon. These platelets had ^{51}Cr

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survival values in vivo about 50% of those observed for fresh autologous platelets.

In another study, platelets isolated from a unit of blood by serial differential centrifugation were frozen in inexpensive polyvinyl chloride plastic bags and in special expensive polyolefin plastic bags: in vitro recovery and in vivo survival values were similar in the two groups. Platelets frozen in the polyolefin plastic bags released ^{14}C-serotonin at increasing concentrations of thrombin at about the same capacity as fresh platelets; the response of platelets frozen in polyvinyl chloride plastic bags was about 90% that of platelets frozen in polyolefin plastic bags.

We are now isolating multiple units of platelets by intermittent flow and by continuous flow centrifugation and freezing them in polyvinyl chloride transfer packs to support thrombocytopenic patients.

Status Report on Cryopreservation of Human Platelets

John J. Vecchione and C. Robert Valeri

ABSTRACT

The equivalent of about 5 single units of platelets was isolated from each of eight normal volunteers by intermittent flow centrifugation using the Haemonetics Model 30 Blood Processor. The platelets were frozen with 6% DMSO in a single polyolefin plastic container in a −80 C mechanical freezer. *In vitro* freeze-thaw-wash platelet recovery values varied with the methods of measurement: 80% when the platelet counts were made by phase microscopy, 85% by Coulter counter, and 60% by the Technicon. These platelets had ^{51}Cr survival values *in vivo* about 50% of those observed for fresh autologous platelets.

In another study, platelets isolated from a unit of blood by serial differential centrifugation were frozen in inexpensive polyvinyl chloride plastic bags and in special expensive polyolefin plastic bags: *in vitro* recovery and *in vivo* survival values were similar in the two groups. Platelets frozen in the polyolefin plastic bags released ^{14}C-serotonin at increasing concentrations of thrombin at about the same capacity as fresh platelets; the response of platelets frozen in polyvinyl chloride plastic bags was about 90% that of platelets frozen in polyolefin plastic bags.

We are now isolating multiple units of platelets by intermittent flow and by continuous flow centrifugation and freezing them in polyvinyl chloride transfer packs to support thrombocytopenic patients.

INTRODUCTION

During the past three years the Naval Blood Research Laboratory has been isolating multiple units of platelets in special cell separation machines and freezing them by the protocol developed for freezing a single unit of platelets isolated by serial differential centrifugation[1-9]. We have studied the methods used to quantitate the freeze-thaw-wash recovery and the *in vivo* circulation of previously frozen platelets, and have re-evaluated the requirements for the special polyolefin plastic freezing bags thought to be superior to polyvinyl chloride transfer packs for platelet freezing[10-12].

This paper reports data on freeze-thaw recovery and freeze-thaw-wash recovery determined with the Coulter counter, Technicon, and phase microscopy methods, on the measurement of ^{51}Cr platelet survival *in vivo*, on the use of polyvinyl chloride plastic containers to freeze platelets isolated from a unit of blood by serial differential centrifugation, and on the cryopreservation of human platelets isolated by intermittent flow centrifugation in the Haemonetics Blood Processor 30.

MATERIALS AND METHODS

Platelet Enumeration and the Calculation of *In Vitro* Freeze-Thaw-Wash Recovery

Platelet enumeration was performed using phase microscopy[13], the Coulter counter*[14], and the Technicon optical system+[15]. When using the Technicon Company reference standard+, we calibrated this machine after we had determined the platelet count of the standard by phase microscopy. Coulter counts were performed using the Model ZBI with a high resolution 50/60 u aperture and an attached H4 channelyzer. The settings were: amplification 1.0, aperture current 0.5, gain 8.5, matching switch 20K, sampling mode 100, lower threshold 5, upper threshold 100; these threshold limits corresponded to a platelet volume range of 1.2 u^3 to 25 u^3. The machine was calibrated with 2.02 u diameter latex spheres*. The platelet sample was diluted 1:20,000 in isoton prefiltered with a 0.22 u^2 Millipore filter‡; background counts were always less than 100. Cell concentration was maintained below 10% to minimize coincidence, and samples were counted in triplicate

*Coulter Electronics, Hialeah, FL
+Technicon Autocounter, Technicon Instruments Corp., Tarrytown, NY
‡Millipore Corp., Bedford, MA

immediately after isoton dilution. When appropriate, isoton was modified by the addition of DMSO to attain the same DMSO concentration as the sample to be counted.

Freeze-thaw-wash recovery value reports the total number of platelets recovered after freezing, thawing, washing, and resuspension, divided by the total number of platelets frozen. Freeze-thaw-wash recovery was calculated so as not to include platelet loss due to sampling during the procedures:

$$\text{Freeze-Thaw-Wash Recovery} = \text{Freeze-Thaw Recovery} \times \text{Thaw-Wash Recovery} \times 100$$

Where

$$\text{Freeze-Thaw Recovery} = \frac{\text{Post-Thaw Platelet Count} \times \text{Volume After Thaw}}{\text{Pre-Freeze Platelet Count} \times \text{Volume Frozen}}$$

And

$$\text{Thaw-Wash Recovery} = \frac{\text{Post-Wash-Resuspension Platelet Count} \times \text{Volume in Which Washed Platelets Resuspended}}{\text{Post-Thaw Platelet Count} \times \text{Volume of Thawed Platelets}}$$

51-Chromium Platelet Survival *In Vivo*

Fresh platelet concentrates, and previously frozen platelets after thawing but prior to addition of wash solution, were labeled as described by Murphy and Gardner[16] with the following minor modifications:

A. The fresh and previously frozen platelet concentrates were incubated with sodium chromate[§] at room temperature for 30 minutes rather than 15 minutes.
B. The fresh platelet concentrate was washed by dilution centrifugation after the rapid addition of 100 ml autologous platelet-poor plasma (PPP) and 10% acid citrate dextrose solution (ACD, NIH, Formula A). The diluted platelets were centrifuged at 4500 g × five minutes, the supernatant expressed as completely as possible, and the cells resuspended in 30 ml PPP. Previously frozen platelets were washed as described below.
C. Platelet-associated ^{51}Cr radioactivity in the injected platelets was determined in duplicate 0.5 ml aliquots following two 7.5 ml washes with 1% ammonium oxalate and one 7.5 ml wash with saline. In each of these dilutional wash steps, the chromated platelets were centrifuged at 7000 × g for five minutes and then resuspended in 0.5 ml volume using a Vortex Genie Mixer[π] just until the platelet pellet was visually resuspended.
D. Two 7.5 ml blood samples, each anticoagulated with 2.5 ml of 1.2% EDTA in saline, were obtained 1 hour, 2 hours, and daily for 7 days after injection of the labeled cells. The platelets were isolated after the blood was centrifuged at 280 × g for five minutes, and then again after 3 ml of saline was mixed with the remaining red blood cells and the centrifugation repeated. Harvested platelets were centrifuged at 7000 × g for five minutes, the supernatant was decanted, and the platelet button was transferred to a gamma tube. The recovery of platelets during the isolation procedure was calculated from phase microscopy platelet counts and volumes of whole blood and isolated platelets[17].
E. Blood volume was calculated from the plasma volume measured with 0.5 uCi iodinated ^{125}I albumin[‡‡] and the total body hematocrit determined from the peripheral venous hematocrit multiplied by the "f" factor of 0.89[18]. The iodinated albumin and the chromated platelets were injected within 30 seconds of each other. Fifteen minutes after injection, plasma samples obtained for determination of the blood volume showed significant ^{51}Cr radioactivity as a result of the free plasma chromium injected with the chromated platelets. Since some of this ^{51}Cr radioactivity was detected in the ^{125}I window, cross-over corrections were performed before plasma volume was calculated.

Separation of the Cellular Components of ^{51}Chromium Labeled Human Platelet Concentrate by an Albumin Density Gradient

The platelet concentrate was isolated in a triple blood pack[**] by serial differential centrifugation from 450 ml of blood anticoagulated with 63 ml of CPD. The blood was centrifuged[++] at 4500 g for 2.5 minutes at 22 ± 2 C to isolate PRP, the PRP was centrifuged at 4500 g to concentrate the platelets, and all but 20-30 ml of supernatant PPP was expressed. After 60 minutes of undisturbed storage at room temperature the platelets were resuspended manually. The resuspended platelet concentrate was labeled with ^{51}Cr as described above. The cellular components of the ^{51}Cr labeled platelet concentrate were separated using preparative velocity sedimentation at unit gravity through an albumin gradient as described by Catsimpoolas and associates[19]. Albumin solutions were prepared in 0.9% sodium chloride buffered with 10mM sodium phosphate to pH 6.5 (PBS).

The ^{51}Cr labeled platelet concentrate was diluted with a solution containing one part 0.9% sodium chloride and 12 parts PPP. One part of platelet concentrate was mixed with between five and 18 parts of the sodium chloride-PPP solution such that the diluted platelet concentrate would layer on the 1% albumin PBS solution which formed the top of the gradient. A 10 ml volume of the diluted platelet concentrate, containing a total of

[§] E.R. Squibb & Sons, Inc., Princeton, NJ
[π] Scientific Products, Evanston, IL
[‡‡] Ames Company, Elkhart, IN
[**] Fenwal Laboratories, Deerfield, IL
[++] RC3 Refrigerated Centrifuge, Ivan Sorvall Co., Norwalk, CT

about 1×10^7 cells, was placed on the gradient. Plasma in the platelet concentrate prior to dilution was labeled with 1.25 uCi ^{125}I-human albumin[††] to permit tracing the distribution of plasma through the effluent fractions of the column.

About 120 fractions of 1.55 ml volume each were eluted from the albumin gradient. Aliquots of each fraction were sampled to determine ^{51}chromium and ^{125}iodine radioactivity and to enumerate cells. Platelet counts were determined with the Coulter ZBI* used as described above. Samples for platelet counts were diluted 1:10 to 1:200 in isoton before counting. Red cells and white cells were identified and enumerated using Neubauer Hemacytometer[§§] counting chambers in undiluted aliquots. Cell identification was validated using the Coulter H4 channelyzer to determine volume distributions.

Platelet Isolation Using the Haemonetics Model 30 Blood Processor

Platelet-rich plasma was isolated from blood anticoagulated with ACD by intermittent flow centrifugation with the Haemonetics Model 30 Blood Processor[¶¶]. The blood was pumped into the 225 ml bowl as rapidly as possible, usually at a flow rate of 60-100 ml/minute. As soon as the platelet band appeared on the shoulder of the bowl, the flow rate was reduced to 40 ml per minute. Actual platelet collection was started when the platelet band had progressed to 1 cm along the shoulder, and collection was continued for 45 seconds after the red cell interface was reached, the latter indicated by red blood cell appearance in the collection line. The flow rate was maintained at 20 ml per minute during platelet collection without venous pressure cuff occlusion on the donor's arm. The volume of PRP obtained averaged 380 ml and varied from 300 to 450 ml. When gross contamination with red blood cells was visible, the PRP was centrifuged[++] at 160 X g for eight to 12 minutes, at 22 ± 2°C, depending on the volume of the PRP.

Prior to centrifugation to remove the red blood cells, the PRP was acidified to a pH of about 6.5 by addition of 7.5% V/V ACD. About 90% of the contaminating red blood cells were removed during this centrifugation procedure. Platelet isolation was greater when the platelets were contaminated with red blood cells and the red blood cells were removed by centrifugation (0.60×10^{11} platelets per pass) than when platelets were collected with avoidance of red blood cell contamination and no centrifugation step was needed to remove red blood cells (0.39×10^{11} platelets per pass, $p < 0.05$).

Efficiency of platelet isolation was determined by dividing the number of platelets collected by the number of platelets which passed through the bowl and were available for collection, where:

$$\text{Total Number of Platelets Available for Collection} = \text{Average of Pre-pheresis and Post-pheresis Donor Blood Platelet Count per ml Blood} \times \text{Volume of Blood Passing Through Collection Bowl in mls}$$

$$\text{Volume of Blood} = \frac{\text{Collection Bowl Size (225 ml)} \times \text{Hematocrit of Blood in Bowl at Completion of Each Pass}}{\text{Average of Pre- and Post-pheresis Donor Blood Hematocrit}} \times \text{Total Number of Pheresis Passes}$$

The hematocrit in the bowl at the completion of each pass was estimated to be about 80%.

Cryopreservation of Platelets Isolated with the Haemonetics Model 30 Blood Processor and Frozen in Polyolefin Plastic Bags

Red blood cell purified PRP was centrifuged[++] at 4500 X g for five minutes to concentrate the platelets, and all but 30 to 50 ml of the supernatant PPP was expressed from the concentrated platelets.

After 90 minutes of undisturbed storage at room temperature, the platelets were resuspended by gentle manual agitation. The volume of platelet concentrate was adjusted to 50 ml by adding the appropriate volume of PPP. A 50 ml volume of dimethylsulfoxide[***] (DMSO) – plasma freezing solution containing 12 parts of DMSO and 88 parts of PPP was prepared by the addition of DMSO to the PPP with manual agitation over 45 to 60 seconds. No special attempt was made to cool the plasma before adding the DMSO which was autoclaved before use for 20 minutes in a 50 ml glass bottle sealed with a teflon cap. Using a venoset microdrip infusion device[+++] to control the flow rate, the DMSO-plasma freezing solution was added to the platelet concentrate over 30 minutes with mechanical agitation at about 180 lateral oscillations per minute using the low speed on a modified Eberbach shaker[†††].

The platelets, with a final concentration of 6% DMSO, were transferred to a 200 cm^2 polyolefin plastic bag[§§§] which was placed in an aluminum container for freezing and storage in a 80°C mechanical freezer. The freezing rates were not controlled but ranged from 1.7 to 2.7 per minute from room temperature to −40°C. The platelets were stored in the frozen

[††] Ames Company, Elkhart, IN
*Coulter Electronics, Hialeah, FL
[§§] "Bright Line," American Optical Corporation, Buffalo, NY
[¶¶] Haemonetics Corporation, Braintree, MA
[++] RC3 Refrigerated Centrifuge, Ivan Sorvall Co., Norwalk, CT
[***] Crown Zellerbach Corp., Camas, WA, Lot #308162; FDA Authorization for use of DMSO; BB-IND 570
[+++] Abbott Laboratories, North Chicago, IL
[†††] Cryogenic Equipment Corp., Buckeystown, MD
[§§§] UCAR 2030-4, Union Carbide Corp., New York, NY

state from 1 to 57 days, with an average of 11 days for 8 studies. The time lapse between isolation of the platelets and transfer to the −80°C mechanical freezer was not more than four hours.

To thaw the platelet concentrate, it was placed in a 42°C water bath agitated vigorously by a mechanical pump for 2.5 minutes. The wash solution, containing 98 ml of autologous PPP, 2 ml of DMSO, and 20 ml of ACD, was added rapidly to the thawed platelets. The autologous PPP used in the wash solution had been obtained after centrifugation to concentrate the PRP on the day of isolation of the platelets and had been stored at −20°C. The mixture of thawed platelets and wash solution was transferred from the polyolefin freeze bag into a 300 ml polyvinyl chloride plastic bag, because high-speed centrifugation was not practical in the polyolefin bag. The platelets were concentrated by centrifugation[++] at 4500 × g for five minutes at 22°C, the supernatant solution was removed, and the platelets were resuspended in 30 ml of PPP. The pH of the washed platelets was 6.55. The thawing and dilution washing processes were performed at room temperature in less than 1 hour.

Residual DMSO in the platelet concentrate was measured by gas chromatography[¶¶¶]. Cultures on blood agar and peptone broth were performed on each fresh platelet concentrate before the addition of DMSO, and after freezing, washing, and resuspension.

Thrombin-Induced Release of ^{14}C-Serotonin *In Vitro* from Human Platelets Frozen in Polyvinyl Chloride Transfer Packs or in Polyolefin Freezing Bags

Using double serial differential centrifugation[9] in a double plasmapheresis triple blood pack[**], each 500 ml unit of blood anticoagulated with 75 ml of ACD was centrifuged as described above to isolate and concentrate the platelets. After 30 minutes of undisturbed storage at room temperature, the platelets from each unit were resuspended manually, pooled, and the volume adjusted to 80 ml by adding the appropriate volume of PPP. A 20 ml volume of this so-called "fresh platelet concentrate" was tested by serotonin release assay. Sixty ml of a solution containing 12 parts DMSO and 88 parts autologous PPP was added to the remaining 60 ml of fresh platelet concentrate as described above. The resulting 120 ml of platelet concentrate was divided in half; 60 ml was transferred to a 200 cm^2 polyolefin freezing bag[§§§], 60 ml to a 300 ml polyvinyl chloride transfer pack[**], and each placed in an aluminum container for freezing and storage in a −80°C mechanical freezer. The units were thawed simultaneously for 3-5 minutes in a 42°C waterbath agitated vigorously by a mechanical pump. Each unit was washed as described above with a solution consisting of 98 ml of a mixture of 20 parts autologous previously frozen PPP and 80 parts 0.9% sodium chloride/0.2% glucose/25 mg/L disodium phosphate solution[**], 2 ml of DMSO and 16 ml ACD. After washing the platelets were resuspended in 30 ml autologous PPP.

The assay of platelet ^{14}C-serotonin release at various concentrations of thrombin was performed as described by Robblee and co-workers[20], except that we used an unmanipulated platelet sample for labeling rather than a washed TRIS-saline-glucose (TSG) resuspended platelet sample. Thrombin was incubated with ^{14}C-serotonin labeled platelets washed once with TSG and resuspended in TSG buffer at a phase microscopy adjusted platelet count of 300,000/mm^3.

Freeze-Thaw-Wash Recovery *In Vitro* and ^{51}Chrominum Survival *In Vivo* of Platelets After Freezing in Polyvinyl Chloride Transfer Packs or in Polyolefin Freezing Bags

Single units of platelet concentrate were isolated by serial differential centrifugation as described above from 450 ml blood collected from normal donors into 63 ml CPD-adenine anticoagulant[**]. (Table 1) Three subtypes of CPD-adenine were used:

CPD-A$_1$ contains 2000 mg glucose and 17.3 mg adenine per 63 ml anticoagulant

CPD-A$_2$ contains 2810 mg glucose and 34.6 mg adenine per 63 ml anticoagulant

CPD-A$_3$ contains 3210 mg glucose and 34.6 mg adenine per 63 ml anticoagulant

The platelet concentrates were frozen either in polyolefin freezing bags[§§§] or in polyvinyl chloride transfer packs[**] and then thawed, labeled with ^{51}chromium, and washed as described above.

RESULTS

Enumeration of Fresh and Frozen-Thawed Platelets Using the Coulter Counter, Phase Microscopy, and the Technicon Optical System

Because platelet counts on fresh samples of whole blood, platelet-rich plasma and platelet concentrate determined with the Technicon optical system were about 30% higher than those obtained with phase microscopy or with the Coulter counter when the Technicon was calibrated with the value indicated on the company reference standard, we calibrated this machine using a platelet count of the standard determined in our laboratory by phase microscopy.

Figure 1 compares platelet counts determined with the Coulter, phase microscopy, and Technicon (phase calibrated) in samples of fresh platelet concentrate after the addition of DMSO, and shows no differences with the three systems. Figure 2 shows highly significant differences in the platelet counts determined after freezing and thawing: Coulter counts were about 20% greater and Technicon counts were about 20% lower than phase microscopy counts, and Technicon counts were only 70% those of Coulter counts. Figure 3 shows that platelet counts with the three systems after the freeze-thaw-wash process were similar to those after freeze-thaw.

Freeze-thaw-wash recoveries calculated from platelet counts were about

[++]RC3 Refrigerated Centrifuge, Ivan Sorvall Co., Norwalk, CT

[¶¶¶]Hewlett-Packard Corp., Lexington, MA

[**]Fenwal Laboratories, Deerfield, IL

[§§§]UCAR 2030-4, Union Carbide Corp., New York, NY

20% higher with the Coulter and about 25% lower with the Technicon than values by phase microscopy, and Technicon values were only about 65% of those seen with the Coulter (Figure 4).

^{51}Chromium Determination of Platelet Survival *In Vivo*

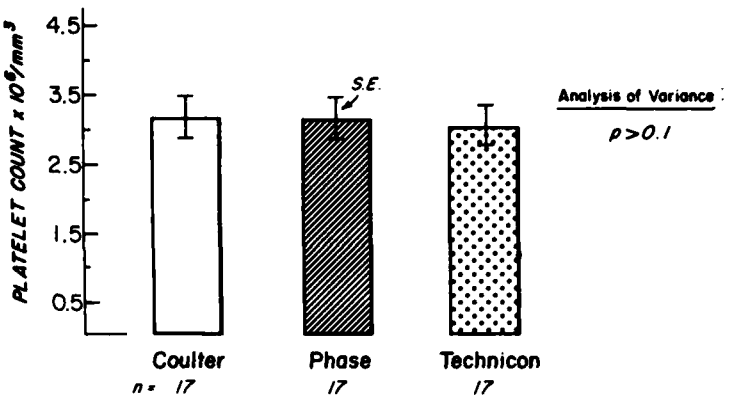
To measure platelet survival *in vivo* by ^{51}chromium labeling, the amount of ^{51}chromium radioactivity that is associated with the injected platelets must be determined. In our laboratory this is done by washing a sample of the injected labeled cells twice with ammonium oxalate and once with 0.9% sodium chloride in an attempt to lyse red blood cells and to remove free plasma ^{51}Cr radioactivity. After each dilution centrifugation wash, the platelet pellet is vortexed to resuspend the cells prior to addition of the subsequent wash solution. We have found that prolonged vortexing during efforts to resuspend the platelet pellet reduces platelet-associated radioactivity and thereby increases platelet survival. To standardize the preparation of those samples, we used clear plastic centrifuge tubes and vortexed only until the platelet pellet was visually resuspended from the bottom of the centrifuge tube.

The accuracy of this technique of determining platelet-associated radioactivity was evaluated by separating the cellular components of ^{51}chromium labeled fresh platelet concentrate using an albumin density gradient (Figure 5).

Mononuclear cells, which constitute over 95% of the white cells, emerged first, followed by red cells, and then by platelets. Plasma, traced through the column using 125-iodinated albumin, appeared in greatest quantity just after the platelet peak. Platelet-associated radioactivity measured by albumin gradient was similar to that measured by our standard procedure.

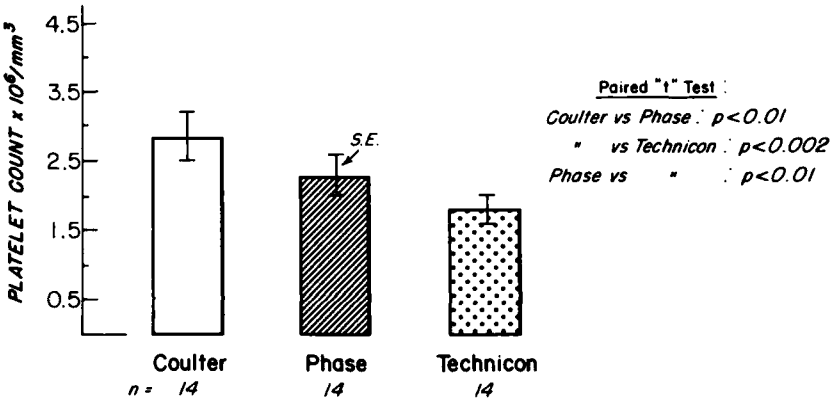
Cryopreservation of Platelets Isolated by Intermittent Flow Centrifugation with the Haemonetics Model 30 Blood Processor and Frozen in Polyolefin Plastic Bags

The total number of platelets isolated during a six-pass pheresis in each



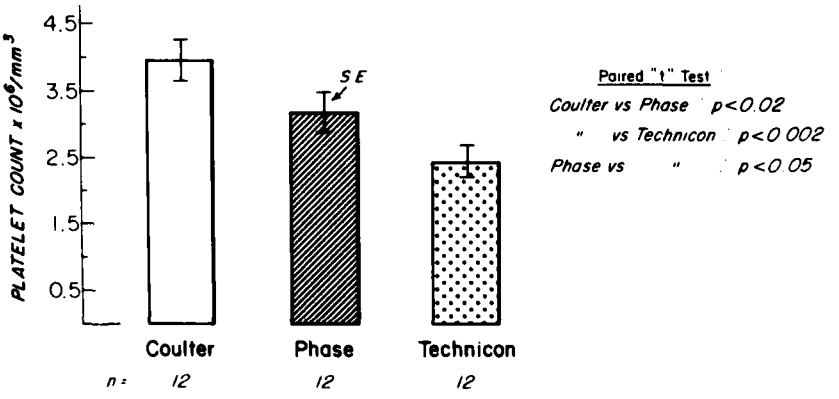
The platelet count in fresh platelet concentrate after the addition of DMSO. Platelet counts were determined using the Coulter counter, phase microscopy, and the Technicon optical system. The Technicon was calibrated after the platelet count of the company standard was determined in our laboratory using phase microscopy.

Figure 1



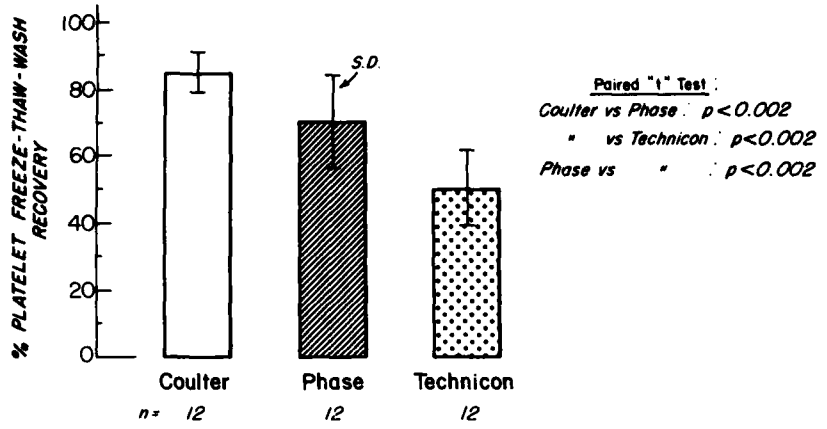
The platelet count in platelet concentrate after freezing and thawing.

Figure 2



The platelet count in platelet concentrate after freezing, thawing, washing and resuspension.

Figure 3



The platelet freeze-thaw-wash recovery calculated from platelet counts measured before freezing, and on the same units of platelets after thawing and washing. Statistically significant differences exist between the Coulter counter, phase, and Technicon values.

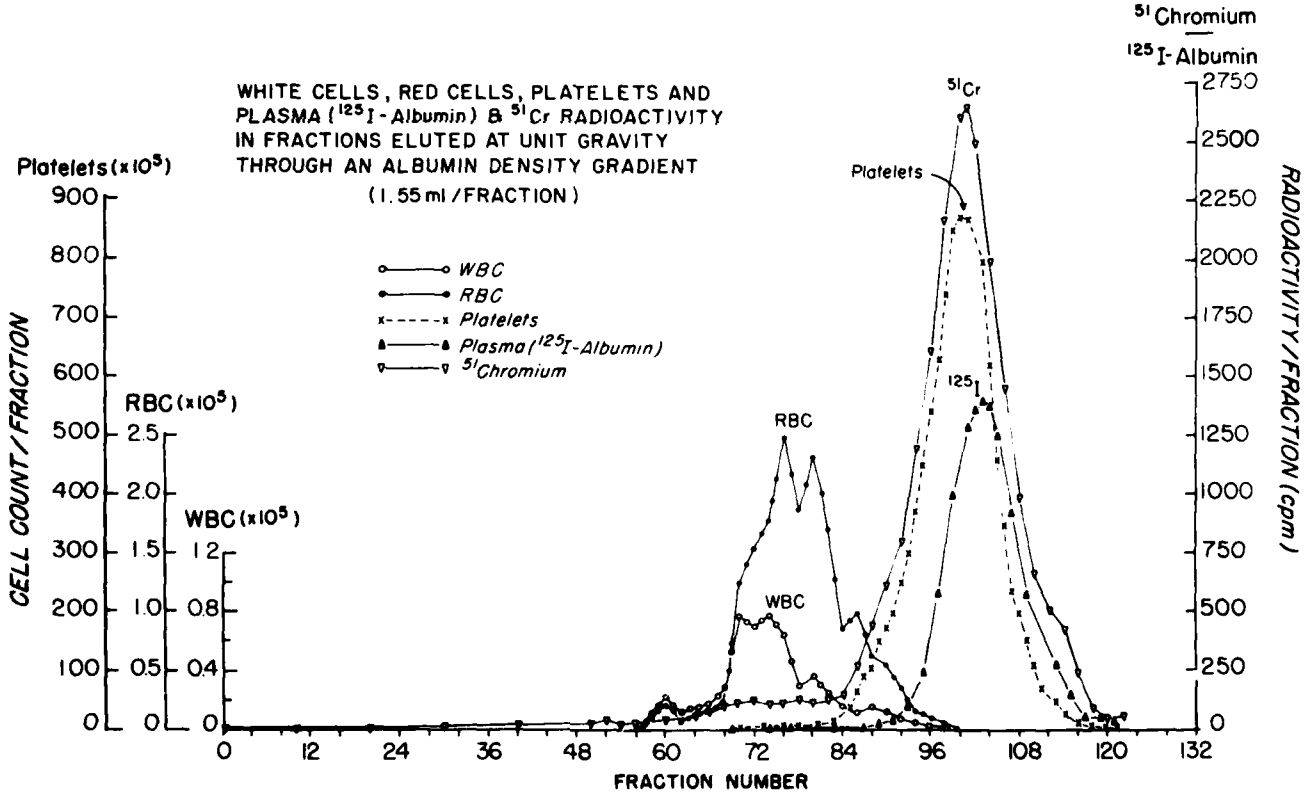
Figure 4

of eight volunteers averaged 4.34×10^{11}; efficiency of platelet isolation was 83%. About 87% of the platelets were recovered after the centrifugation steps to remove red blood cells and concentrate the platelets.

The freeze-thaw-wash recovery values were similar to those seen in platelets isolated from single units: about 85% with the Coulter counter[1-5,8], slightly lower with phase microscopy and considerably lower with the Technicon optical system. (Figure 6).

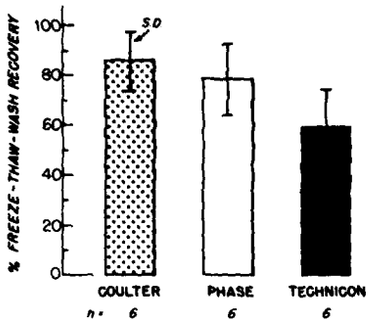
In vivo survival values of the previously frozen platelets determined using ^{51}Cr are presented in Figure 7; the average *in vivo* recovery value between 1 and 2 hours after transfusion was about 32%; the *in vivo* recovery was about 50% that of fresh platelets.

In none of the platelet units studied did bacterial growth occur. The residual amount of DMSO in four studies



Fresh platelet concentrate was labeled with ^{51}chromium and ^{125}I-albumin and applied to an albumin density gradient. The white blood cells, red blood cells, platelets, and plasma components were separated.

Figure 5



The freeze-thaw-wash recovery of cryopreserved platelets isolated from healthy volunteers by intermittent flow centrifugation using the Haemonetics Model 30 Blood Processor. Platelet counts were performed using the Coulter counter, phase microscopy, and the Technicon optical system.

Figure 6

after washing and resuspension was 334 mg; DMSO washout efficiency was about 92%.

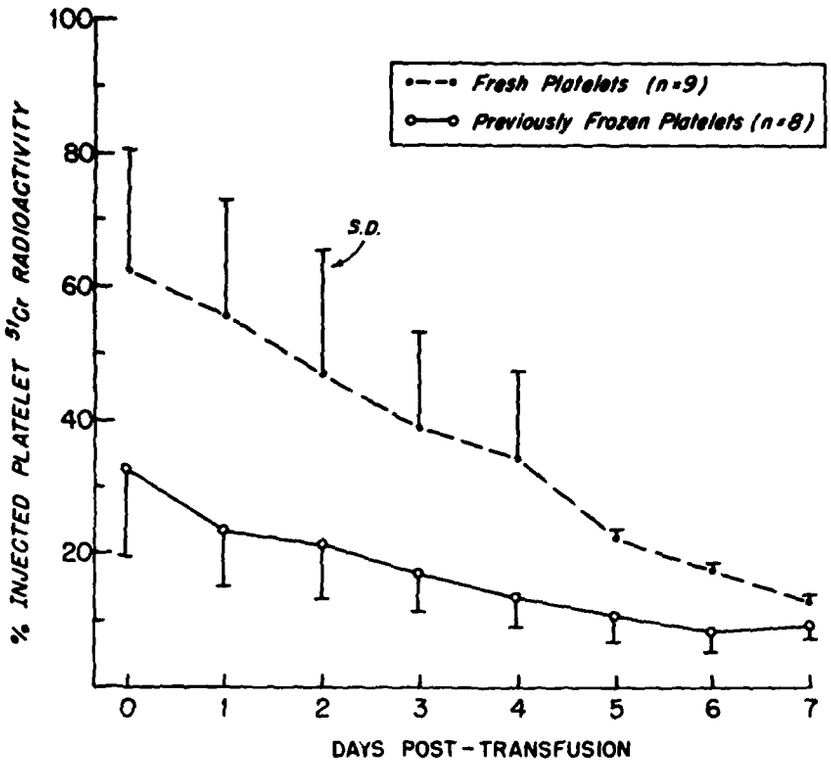
Cryopreservation of Platelets Isolated by Serial Differential Centrifugation and Frozen in Polyvinyl Chloride Transfer Packs or Polyolefin Freezing Bags

A. *In Vitro* Function

Figure 8 compares the thrombin-induced release of ^{14}C-serotonin from fresh platelet concentrates and from platelets frozen in polyolefin freezing bags and in polyvinyl chloride transfer packs. Previously frozen platelets released more serotonin than did fresh platelets at the lowest concentration of thrombin studied: this difference was significant for the platelets frozen in polyolefin compared to fresh platelets at a thrombin concentration of 0.048 u/ml. The ^{14}C-serotonin release from platelets frozen in polyvinyl chloride was about 90% that of platelets frozen in polyolefin; this difference was significant at each of the five thrombin concentrations studied.

B. *In Vitro* Freeze-Thaw-Wash Recovery

Table 1 shows the freeze-thaw-wash recovery of platelets frozen in polyvinyl chloride transfer packs and in polyolefin plastic bags. These platelets were isolated by serial centrifugation from units of blood anticoagulated with CPD-A$_1$, CPD-A$_2$, or CPD-A$_3$, and the platelets were counted by both phase microscopy and the Technicon. Freeze-thaw-wash recovery using phase microscopy was about 85% and using the Technicon about 65%, whether the polyolefin or polyvinyl chloride plastic container was used.

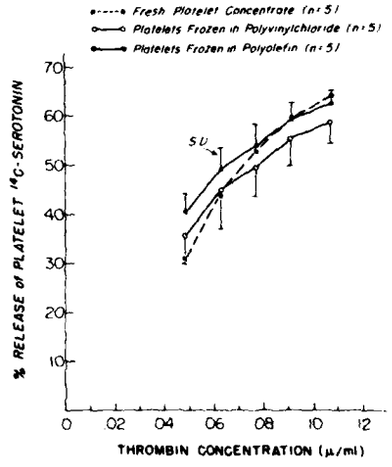


The ^{51}Cr survival of previously frozen platelets isolated by intermittent flow centrifugation compared to fresh platelets isolated by differential serial centrifugation in normal volunteers.

Figure 7

C. ^{51}Chromium Survival *In Vivo*

Table 1 also compares the ^{51}Cr survival of autologous platelets frozen in polyvinyl chloride transfer packs and polyolefin plastic bags. Overall recovery of platelet radioactivity between one and two hours after transfusion was about 40%. The type of freezing bag did not affect the immediate recovery or the lifespan of the platelets *in vivo*.



The thrombin-induced release of ^{14}C-serotonin from fresh platelet concentrate and from washed platelets previously frozen in a polyvinyl chloride transfer pack or in a polyolefin plastic bag.

Figure 8

Table 1. Freeze-Thaw-Wash Recovery and ^{51}Cr Survival of Platelet Concentrate Frozen in Polyvinyl Chloride Transfer Packs or in Polyolefin Freezing Bags.

CPD-Adenine Subtypes								
Anticoagulant	**Polyvinyl Chloride**				**Polyolefin**			
	Freeze-Thaw-Wash Recovery		**^{51}Cr Survival**		**Freeze-Thaw-Wash Recovery**		**^{51}Cr Survival**	
	Phase	**Technicon**	**Initial Recovery**	**$T^{1/2}$ (days)**	**Phase**	**Technicon**	**Initial Recovery**	**$T^{1/2}$ (days)**
CPD-A_1	90%	75%	45%	3.7	69% (58-79)	65% (58-70)	26 (24-27)	3.9
	N=1	N=1	N=1	N=1	N=2	N=2	N=2	N=2
CPD-A_2	95% (90-100)	73% (72-73)	35% (29-41)	4.3	81% (71-93)	68% (61-79)	39%	4.2
	N=2	N=2	N=2	N=2	N=3	N=3	N=1	N=1
CPD-A_3	94% (86-105)	71% (66-81)	44% (17-58)	4.5	89% (88-90)	60%	56% (43-65)	4.5
	N=5	N=4	N=5	N=5	N=2	N=1	N=2	N=2
All CPD-Adenine	94% (86-105)	72% (66-81)	42%±11.5%* (17-58)	4.4	80% (58-93)	65% (58-79)	39%±17.2%* (24-65)	4.2
	N=8	N=7	N=8	N=8	N=7	N=6	N=5	N=5

( ) indicates range
*indicates mean ± 1 standard deviation

DISCUSSION

Multiple units of human platelets isolated from normal volunteers using intermittent flow centrifugation in the Haemonetics Blood Processor 30 have been successfully frozen with 6% DMSO in special polyolefin plastic containers and stored at −80°C for at least two months. Although studies on the function of these platelets have not been completed, *in vitro* recovery and *in vivo* survival values were found to be similar to those observed when human platelets isolated from a single unit of blood were frozen with 4-5% DMSO and stored at 80°C for at least 8½ months[6].

An important criterion in evaluating the quality of cryopreserved human platelets is the cell recovery *in vitro* after the freeze-thaw-wash procedure, and this measurement is influenced by the platelet counting method. Phase microscopy gives the most accurate measurement; the Coulter counter yields higher platelet counts probably because other particles are counted with the platelets; and the Technicon counter yields lower measurements. When fresh platelets were counted, results were similar with the three methods.

The radionuclide ^{51}Cr can be used to measure the *in vivo* survival of fresh and preserved platelets, that is the immediate recovery, and the life span of the platelets after transfusion. When autologous platelets are transfused, the immediate recovery usually reveals the damage to platelets as a result of preservation injury. Using ^{51}Cr to determine the survival of platelets presents a technical difficulty since it is necessary to establish what amount of platelet-associated radioactivity was injected. The recovery of the platelet-associated radioactivity after the transfusion depends on the total amount of radioactivity associated with the infused platelets, the blood volume of the recipient, and the number of platelets isolated when blood samples are obtained after platelet injection. By using an albumin gradient cell separation procedure, we have been able to confirm that our method affords an accurate determination of the infused platelet-associated radioactivity. We recommend that measurements of fresh autologous ^{51}Cr platelet survivals should be performed when possible in the same individual in whom ^{51}Cr freeze-preserved autologous platelet survivals are performed to control the ^{51}Cr platelet survival procedure.

We have demonstrated that platelets isolated from a single unit of blood can be satisfactorily frozen in the inexpensive polyvinyl chloride plastic transfer packs which are integrally attached to the primary collection bags. Our *in vivo* data on the one to two hour post-transfusion recovery and lifespan values are similar for platelets frozen in polyvinyl chloride plastic bags and polyolefin plastic bags. The *in vitro* test that we utilize to measure platelet function is the